EXHIBIT UU

DOCKET 100/150

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
)
SHMUEL CABILLY ET AL.)
)
Serial No. 06/483,457)
)
Filed: April 8, 1983)
)
For: RECOMBINANT IMMUNOGLOBIN)
PREPARATIONS)

Art Unit: 127

Examiner: J. HULEATT

AFFIDAVIT UNDER C.F.R. § 1.131

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, RONALD B. WETZEL, being duly sworn, depose and say that I am a coinventor of the subject matter claimed in the above-described patent application. Affiant further deposes and says that the subject matter of at least claims 53-56, 58-60 and 63-67 was reduced to practice in the United States of America prior to March 25, 1983, as shown in the attached Exhibits. The specific dates appearing in the Exhibits have been obscured.

Exhibit 1 is a Western blot showing the levels of stable expression of murine anti-CEA gamma and kappa immunoglobulins (and a mixture of gamma and kappa) in E. coli transformed with plasmids bearing genes encoding the gamma, kappa, and gamma and kappa immunoglobulin chains, respectively. The levels of each of the proteins are shown in

LC8x076.mdh

PLAINTIFF
EXHIBIT 66
2:08cv03573

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Cabilly Exhibit 2170
Cabilly v. Boss

the table at the top of the Exhibit.

Exhibit 2 is a representative example of how the bacterially-produced immunoglobulin chains were refolded into an immunologically active form, i.e. a form in which they were able to bind to CEA. Column D is a control (E. coli transformed with interferon), while columns A, B and C, respectively, represent refolding experiments on an extract from E. coli transformed with two plasmids, each bearing one of the gamma or kappa chain DNAs (A), an extract from E. coli transformed with a plasmid bearing the kappa chain only (B), and a combined extract from E. coli transformed with a plasmid bearing the gamma chain only (designated "43C") and a plasmid bearing the kappa chain only (C). Column D is a control. As expected, the results with the cotransformant extracts and combined extracts were essentially the same, in both cases indicating anti-CEA activity on the part of the refolded immunoglobulins (576 and 454 versus the control level of 2), while the refolded kappa chain, not having the companion variable region from the heavy (gamma) chain, was considerably less active.

Exhibit 3 is a similar experiment, although it includes additional runs with variations in the refolding reagents and conditions. Again, columns A and G-J show that the refolded extract from kappa and gamma cotransformed E. coli immunologically binds to its specific antigen, CEA, as does the refolded combined extract from separately

LC8x076.mdh

transformed E. coli (column C). Consistent with the results in Exhibit 2, refolded kappa chain was less active. Refolded recombinant gamma chains (column E) also were less active than the combined chains.

Further deponent sayeth not.

Ronald B. Wetzel

Ronald B. Wetzel

7/22/86

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EXHIBIT 1

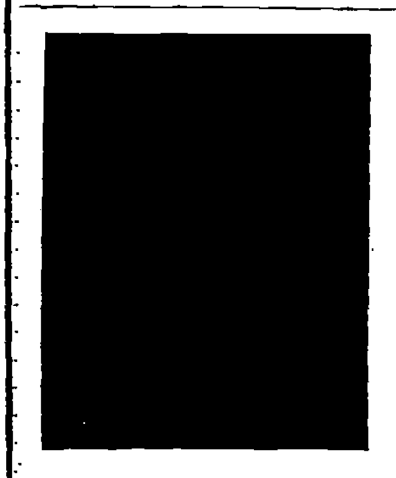
TITLE Expression level

Project No.______
Book No.______ 59

From Page No.___

Jamie's Western blot indicates that, per 2 μg w/ cells, there are the following levels of stable expression

		per
γ cells –	~ 1 μg γ	.5 ng
κ cells –	~ .3 μg	1.5 ng
γ, κ cells –	~ .1 μg κ, .2 μg γ	.5, 1.8 ng (κ, γ)



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GENE-CEN 012642

EXHIBIT 2 p.1

Project No.
Book No.
TITLE Use best conditions (pg 74) with Fresh E coli; ~~Stock~~ [illegible]

78

From Page No. — neurea, 5M urea, 5M guanidine, glycerol?

	A	B	C	D
F,K extract	100			
K extract		100	100	
43 C			25	
IFN extract				100
urea	500	500	500	500
2 [illegible] BME	10	10	10	10
1M tris 8.5	50	50	50	50
250mM EDTA	4	4	4	4
	~1000			

Dialyze against 4°, 5M urea, pH 10.8 .1M Na glycinate, [illegible] 5mM GSH, 0.1 mM GSSG, 10mM EGTA

Dil to 1st ... to PBS overnight

Results (mg/ml)

	A	B	C	D
undiluted:	576	43	454	2
1→5, PBS, 4°:	425	60	570	10
1→5, BSA, ":	410	45	445	15

F,K extract: (100)(576−43) / 200,000 = 0.27%

K extract: (100)(454−43) / 600,000 = .07%

Best reconstitution yet from coli extract. May be a low estimate because of loss of F,K in extract or loss of IgG during dialysis.

* estimate from Western of yield in SDS treated frozen cells, pg. 59

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TITLE Cleck

From Page No. 74

54 / 200 ml / 43C / 1 M tris / 250 mM / ~~sodium~~ / Na

Dialy.

Dialy:

total 30 µl

Witnessed & Unde[rstood] [signature]

EXHIBIT 2 p. 2

Project No.
Book No. 79

TITLE Check variation on pg 74 but without

From Page No. 74

	A	B	C – CEA 1→20 PBS (64A)
54B	150	120	
200 mM phos	20	15	
43C	10	10	
1 M tris 7.5	20	10	
250 mM EDTA	1	1	
~~sulfate~~	~~—~~		
Na 8M urea	—	135	

Dialyze B against same buffers as pg 78

Dialyze 70 µl of A — 79Aa
70 µl of A against no urea — 79Ab
70 µl of A against .5 M gua — 79Ac

Took 30 µl of 79Ab labelled EW79Ab1
79Ac — EW79Ac1
79B — EW79B1
79 [illegible] — EW79[illegible]

[illegible] of 79C from freezer into PBS labelled [illegible]

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GENE-CEN 012644

EXHIBIT 3

80

Project No.
Book No.
TITLE Repeat Exp. pg. 78 exactly

From Page No. 78

	A	B	C	D	E	[illegible]	[illegible]
Y, K extract	650	-	-	-	-	65	32
K extract	-	650	650	-	650	325	98
Y extract	-	-	25	-	-	75	
43C	-	-	-	650	-		
FN extract	-	-	-	-	-		
solid urea, mg	500	500	500	500	500		
2M BME	10	10	10	10	10		
1M tris 8.5	50	50	50	50	50		
250 mM EDTA	4	4	4	4	4		
potential IgG µg/ml	114	0	225		0		

1) Start 2 A dialyses. To one, during transfer to PBS dialysis, add 50µl of 10mg/ml BSA.

PBS dialysis: include aCEA & BSA dialysis as control
Z: 54A 1→20 in PBS
Y: 54A 1→20 in PBS/BSA
0.5 g/ml
X: 54A 1→20 in PBS, [illegible]

In 4° PBS, 1mM PMSF, 5 [illegible] →
also 8 series

ng/ml	A	B	C	D	E	F	G	H	I	J	K	L	M	N	X Y Z
unclotted	109 / 56	53	655	14	56	650	560	288	1172	214	850	55	614	60	> [illegible]
diluted, conc	59 / 552	108	98	0	441	829	1214	353	154	935	1019	171	985	130	[illegible]
2 gold:	.76	.32					1.25	.98	1.54	1.09					

Conclusions:
(1) lower concentration facilitates refolding
(2) addition of BSA to second (PBS) dialysis keeps IgG in soln (.5 g/ml)
(3) .5M urea helps at pH 10.8
(4) 25°C hurts; extra GSG doesn't help
(5) [illegible]

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Date

TITLE More
From Page No. 74
IK:
solid urea
2 M BME
1 M tris
250 mM EDTA
Soln
81 Stock
pH 10.8 1M
8M urea, 50mM
8M [illegible], 50mM
guanidine
Dialyze I
Dialyze E
K
L
M
N
Next:
Witnessed & Understood

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GENE-CEN 012645

EXHIBIT 3

Project No.
TITLE More optimization, non on ?, K extract. Book No. 81

From Page No. 74

81 Stock — RT, dialysis
conc's
?? 1000 — pH 11 & reduction
solid urea ?250
2M βME 15
1M tris 75
250 mM EDTA 6

Soln		F	G	H	I	J
81 Stock	—	200	100	40	10	40
pH 10.8 1M	—	20				
8M urea, 50mM tris 8.5, 1mM EDTA			100	100	100	
8M ???, 50mM tris 8.5, 1mM EDTA						100
potentiate ISG + ??			97	59	10	59

Dialyze A–E against same buffer as pg. 80.

Dialyze 81 Stock against: ______ (i.e., no urea)

K pH 10.8 glycine, .1M, 5mM GSH, .1mM GSSG, 4°

L " " " , 25°

M pH 10.8 glycine, .1M, 10mM GSH, 1mM GSSG, 4°

N 1% triton

Next: repeat coli extracts
1) diluted 1→10
2) added BSA between dialyses (or at beginning)

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(Left margin notes:) M N X Y Z; 614 60; 985 130; → 20 in PBS; → 20 in PBS/BSA; 0.5 mg/ml; ?? 50 μl of; ?? (.5 mg/ml)

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GEN-CEL 0450

GENE-CEN 012646